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Skin Reactivity in Guinea Pigs Sensitized to Flea Bites: The Sequence of Reactions.* (27040)

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It is generally recognized that both humans and animals manifest various types of skin response to bites of hematophagous insects. Some individuals fail to react to bites, some have delayed or immediate skin reaction, while others show both immediate and delayed skin responses. Repeated exposure of an individual to bites of a given species ultimately may completely alter the type of reactivity(1-4,6). Mellanby(3), on the basis of limited clinical observations on humans has shown that, following repeated exposures over varying periods of time, allergic skin responses to mosquito bites are manifested by delayed skin reactions followed by the immediate type. He presented the hypothesis that the skin response of man to mosquito bites occurs in 4 distinct stages: 1) delayed, 2) delayed and immediate, 3) immediate only, 4) non-reactive. However, no experimental evidence was presented for the development of the state of non-reactivity. Generally, in hypersensitivity involving delayed skin reactions, little is known about hyposensitization or desensitization following repeated introduction of antigen. For example, equivocal success has been achieved in desensitization to tuberculin (8). On the other hand, depending on the desensitizing dose, desensitization of guinea pigs to protein antigens has been achieved for periods not exceeding 10 days(9).

The following study indicates that a definite sequence of types of reactivity occurs in guinea pigs in response to repeated exposure to flea bites, and that this sequence terminates in a stage of non-reactivity which lasts for at least 5 months.

Experimental. Three groups of guinea pigs (5 per group) were sensitized to flea bites by daily exposure of each animal to bites of 20 cat fleas, *Ctenocephalides felis felis* (Bouche) according to the method described by Benjamini, Feingold, and Kartman(6). When delayed reactions appeared approximately 5-6 days after the initial exposure, the animals were further exposed twice per week with few exceptions (Table 1) for about 14 weeks. One group was exposed to bites of 20 fleas per

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animal; a second group to bites of 200 fleas; and the third group was exposed once to bites of 20 fleas and once to bites of 200 fleas during the same week. Each animal was observed for skin reactivity at 20 minutes, 1 hour, 4 hours and 24 hours following the exposure. Immediate reactions were considered those which appeared within 4 hours; reactions which appeared after 18-24 hours were classified as delayed.

During the course of the work the number of animals used decreased due to traumatic effects of cardiac puncture and natural causes (the actual number of animals available for each challenge is shown in Table 1. Note that during the period of sensitization through July 23, the control animals are included in the figures).

Controls consisted of 4 additional groups of 5 animals per group which were sensitized to flea bites by daily exposure of each animal to 20 fleas. At the end of 5-7 days, these animals reacted with delayed skin reactions which appeared 18-24 hours following the bite. After 2-5 additional days of exposure to bites of 20 fleas, the animals reacted with both delayed and immediate skin reactions, the immediate skin reactivity appearing 30-60 minutes following the bite. At this point, exposures of the 4 control groups were stopped except for challenges with bites of 20 fleas per animal which were given as follows: one group at 3 months, one at 6 months, another at 12 months and the last group at 18 months following initial exposure.

Results and Discussion. Since no significant variation in reactivity occurred in the 3 groups of experimental animals (those which were exposed to 20, 200, or 20 and 200 fleas twice a week, respectively), the data from these groups were pooled.

The data presented in Table 1 clearly demonstrate the occurrence of a definite sequence in type of response of guinea pigs to bites of fleas. The characteristic lesions involved in delayed or immediate skin responses to flea bites have been previously described(6). During the first 3-4 days of exposure only a few animals reacted. Most animals showed delayed skin reactivity after 5 days of exposure, the reactions appearing 18-24 hours following the bite. In all, a period of 5-6 days was required for most animals for initiation of hypersensitivity. With continued exposure to the antigenic stimulus (flea bites) the animals responded with immediate reactions which developed into the delayed type. The immediate reactions appeared approximately 9 days following initial exposure (2-5 days after appearance of the delayed reactions) and became prominent within 20 minutes to 1 hour following the challenging bite. During a period of approximately 7 weeks, most animals exhibited both delayed and immediate skin reactions; however, the skin reactivity of some animals alternated between delayed only and delayed plus immediate. Toward the end of this period, the percentage of animals showing both types of reactions decreased, while the percentage of those with solely immediate reactivity increased sharply. Thus, the reactivity entered a new phase characterized by only immediate skin responses appearing within 20 minutes to 1 hour following the bite and completely fading within 4 hours. This phase lasted approximately one month, after which time the majority of animals became non-reactive.

In contrast to the test animals (see above) all control animals which were challenged at 3, 6, and 12 months following the initial exposure responded to the challenging bites with both immediate and delayed skin reactions. Of the 5 animals in the control group which were challenged 18 months following the initial exposure, 2 reacted with both immediate and delayed reactions, one showed delayed reactions only and the remaining 2 guinea pigs died before they could be tested. These results indicate that without repeated exposures, the hypersensitive state persisted at least 12-18 months.

The state of non-reactivity of the experimental animals was very likely the result of repeated exposure of the animals to the material introduced by the flea bites. With the establishment of a condition of relative desensitization it was of interest to determine the duration of this state. To test this, starting on Nov. 3, the guinea pigs were challenged periodically with bites of 20 fleas each on the dates shown in Table 1. It was found that the period of non-reactivity lasted for at least 5 months.

Summary. Experimental evidence is presented which demonstrates the occurrence of a

TABLE I. The Sequence of Skin Reactivity of Guinea Pigs to Flea Bites Following Repeated Exposures.

Reactivity Stage	Date of Exposure	No. of Animals Used	Delayed Reactions Only No. Reacting	Delayed Reactions Only % Reacting	Delayed and Immediate Reactions No. Reacting	Delayed and Immediate Reactions % Reacting	Immediate Reactions Only No. Reacting	Immediate Reactions Only % Reacting
	July 16, 1960	30	2	6.7	0	.0	0	.0
Stage I	17	30	2	6.7	0	.0	0	.0
Induction	18	30	3	10.0	1	3.3	0	.0
	19	30	8	26.6	4	3.3	0	.0
	20	30	22	73.4	3	10.0	0	.0
Stage II	21	25	18	72.0	2	8.0	0	.0
Predominantly	22	25	9	36.0	7	28.0	0	.0
Delayed	23	30	23	76.6	3	10.0	0	.0
	24	14	0	.0	8	57.1	4	28.6
	25	14	6	42.8	6	42.8	2	14.3
	Aug. 1	14	9	64.2	5	35.7	0	.0
Stage III	8	12	2	16.7	4	33.3	4	33.3
Delayed	15	12	12	100.0	0	.0	0	.0
and	19	12	1	8.3	11	91.7	0	.0
Immediate	22	12	8	66.6	0	.0	0	.0
	25	12	5	41.6	6	50.0	0	.0
	29	12	6	50.0	0	.0	0	.0
	Sept. 1	12	8	66.6	0	.0	0	.0
	6	12	1	8.3	3	25.0	1	8.3
	8	11	0	.0	5	45.5	4	36.4
	12	12	0	.0	4	33.4	0	.0
	16	12	1	8.3	1	8.3	4	33.3
	20	12	1	8.3	3	25.0	6	50.0
Stage IV	22	12	0	.0	3	25.0	7	58.3
Predominantly	26	12	0	.0	0	.0	10	83.4
Immediate	29	12	0	.0	0	.0	12	100.0
	Oct. 4	12	0	.0	0	.0	12	100.0
	6	12	0	.0	0	.0	12	100.0
	11	12	0	.0	0	.0	11	91.7
	13	12	0	.0	1	8.3	6	50.0
	17	12	0	.0	0	.0	5	41.7
	21	11	0	.0	0	.0	0	.0
	27	11	1	9.1	0	.0	0	.0
Stage V	Nov. 3	11	0	.0	1	9.1	1	9.1
Non-Reaction	10	11	0	.0	1	9.1	1	9.1
	17	11	0	.0	0	.0	4	36.4
	Dec. 1	10	0	.0	0	.0	0	.0
	Jan. 3, 1961	10	0	.0	0	.0	0	.0
	Feb. 1	10	0	.0	0	.0	0	.0
	April 17	9	0	.0	0	.0	0	.0

definite sequence of events in the skin reactions of guinea pigs following repeated exposure to flea bites. The sequence was: I—induction of sensitivity; II—predominately delayed skin reactivity; III—delayed and immediate responses; IV—predominately immediate reactivity; V—non-reactivity.

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